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| 1. Your reference | P67034 |
| 2. Patent application number *(The Patent Office will fill in this part)* | **9816039.3** |
| 3. Full name, address and postcode of the or of each applicant *(underline all surnames)* | University College Cardiff Consultants Limited<br>University of Wales Cardiff<br>P O Box 923<br>Cardiff CF1 3TE |
| Patents ADP number *(if you know it)* | 6829675001 |
| If the applicant is a corporate body, give the country/state of its incorporation | |
| 4. Title of the invention | Molluscicidal Composition |
| 5. Name of your agent *(if you have one)* | Urquhart-Dykes & Lord |
| "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* | Alexandra House<br>1 Alexandra Road<br>Swansea SA1 5ED<br>United Kingdom |
| Patents ADP number *(if you know it)* | 1644 005 |

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
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| Number of earlier application | Date of filing *(day / month / year)* |
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| | |

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
*a) any applicant named in part 3 is not an inventor, or*
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| Statement of inventorship and right to grant of a patent *(Patents Form 7/77)* | |
| Request for preliminary examination and search *(Patents Form 9/77)* | |
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11. I/We request the grant of a patent on the basis of this application.

Signature Urquhart-Dykes & Lord    Date

Urquhart-Dykes & Lord    22 July 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr H.W. Austin
01792 - 474327

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Molluscicidal Composition

The present invention is concerned with a molluscicidal composition, a process of preparing the composition and the use thereof.

Slugs and snails are major pests in arable crops; particularly the field slug *Deroceras reticulatum*, with its ability to hollow grain and destroy newly emerged shoots and leaves. Current control measures rely primarily on the use of baited pellets containing a molluscicide, such as methiocarb. Although such pellets have been effective due to the toxicity of molluscicides, such as methiocarb, the latter has also been implicated in the poisoning of non-target organisms. This has been particularly problematic where the non-target organisms are themselves involved in pest control. For example, non-target insects, such as carabid beetles, which are known to be important natural control agents for several crop pests (including slugs), have been know to have died following ingestion of such methiocarb-containing pellets.

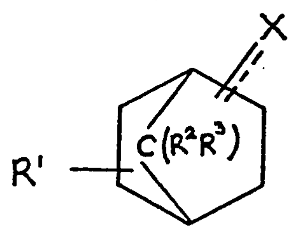
There does, therefore, exist a need for improved, more specific molluscicidal compositions palatable only to molluscs, and the present invention provides such improved compositions, processes of preparation and uses thereof.

According to the present invention there is provided a molluscicidal composition comprising at least one molluscicide for target molluscs, and at least one deterrent for non-target invertebrates, wherein the deterrent is incorporated in the composition so as to have substantially no effect on target molluscs feeding on the composition and to substantially preclude feeding on the composition by the non-target organisms.

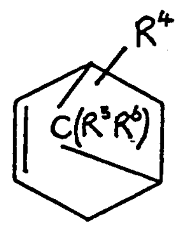
The term "molluscicide" as used herein denotes an agent for destroying molluscs, and typically a composition according to the present invention is employed in destroying slugs, such as slugs of the species *Deroceras reticulatum*. Suitable molluscicides for use in the present invention include methiocarb or thiodicarb, although other available molluscicides can be used. Preferably, methiocarb is employed as a molluscicide in the present invention.

The term "deterrent" as used herein typically denotes a substance that can elicit avoidance behaviour in the non-target invertebrates, through detection of volatile or non-volatile chemicals via receptors on antennae, palps or other mouthparts of the non-target invertebrates. The deterrent employed in the present invention is selected so as to have substantially no effect on feeding molluscs, in other words mollusc feeding is substantially uninhibited by the inclusion of the deterrent in the composition. In this way, the present invention provides a molluscicidal composition that is unpalatable to the non-target invertebrates, but palatable to the target molluscs, and as such is substantially specific for the target molluscs. The non-target invertebrates are preferably insects (such as carabids).

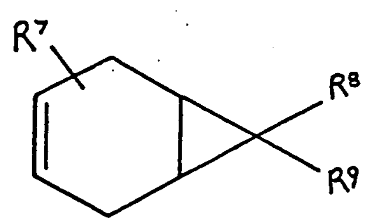
According to a first aspect of the present invention, the deterrent comprises a bicyclic compound. Suitable bicyclic compounds can be represented by any of formulae (I), (II), (III) or (IV):



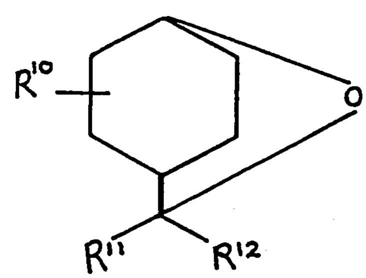
(I)



(II)



(III)

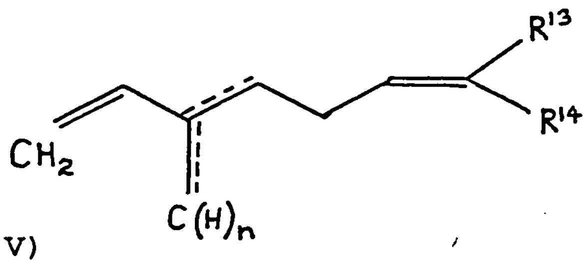


(IV)

where $R^1$, $R^2$, $R^3$, $R^4$ $R^5$, $R^6$, $R^7$, $R^8$, $R^9$, $R^{10}$, $R^{11}$ and $R^{12}$ each independently represent hydrogen or $C_{1-4}$ alkyl, X represents oxygen or hydroxy and ----- represents a bond in the case where X represents oxygen.

Preferred compounds for use as a deterrent according to the first aspect of the present invention include camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one), borneol (*endo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol), eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), α-pinene (2,6,6-trimethylbicyclo[3.1.1]hept-2-ene), D-3-carene (3,7,7-trimethylbicyclo[4.1.0]hept-3-ene) or the like.

According to a second aspect of the present invention, the deterrent comprises a monohydric or polyhydric $C_{6-10}$ alcohol or a compound of formula (V), (VI) or (VII)



(V)

$$\begin{array}{c} \quad Z \qquad\qquad\qquad\qquad\quad R^{16} \\ \quad | \qquad\qquad\qquad\qquad\quad\;\; | \\ R^{15}\text{-}C(H)_m\text{-}Alk\text{-}CH{=}C\text{-}R^{17} \end{array}$$

(VI)

$$\begin{array}{c} \qquad\qquad R^{18} \qquad\qquad\quad R^{19} \\ \qquad\qquad | \qquad\qquad\qquad | \\ HO\text{-}CH_2\text{-}C(H)_p\text{-}C\text{-}Alk_a\text{-}CH{=}C\text{-}R^{20} \end{array}$$

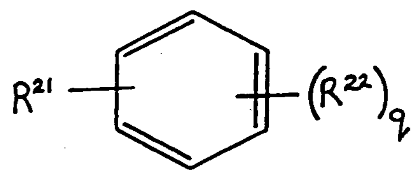
(VII)

where $R^{13}$, $R^{14}$, $R^{15}$, $R^{16}$, $R^{17}$, $R^{18}$, $R^{19}$ and $R^{20}$ each independently represent $C_{1-3}$ alkyl, Alk and $Alk_a$ independently represent a $C_{1-3}$ alkylene chain, Z is oxygen or hydroxy, n is 2 or 3, m is 0 or 1, p is 1 or 2 and ------ represents the optional presence of a bond.

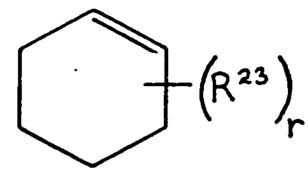
Preferred deterrents for use according to the second aspect of the present invention include one or more of 2-ethylhexane-1,3-diol, citronellol (3,7-dimethyl-6-octen-1-ol), octan-1-ol, β-myrcene (7-methyl-3-methylene-octa-1,6- diene), nerol (*cis*-3,7-dimethyl-octa-2,6-dien-1-ol), β-ocimene (3,7-dimethyl-octa-1,3,6-triene), sulcatol (6-methyl-5-hepten-2-ol) and sulcatone (6-methyl-5-hepten-2-one). More particularly, sulcatol and sulcatone are especially preferred deterrents for use according to the second aspect of the present invention.

According to a third aspect of the present invention, the deterrent comprises a substituted 6-membered carbocyclic ring, where the ring is either benzene or cyclohexene, and where the substituents are one or more of $C_{1-4}$ alkyl, $C_{2-4}$ alkenyl, $C_{1-4}$ alkoxy, hydroxy, $CO_2C_{1-4}$ alkyl, $CON(C_{1-4}$ alkyl$)_2$ and $C_{1-4}$ alkyleneCON($C_{1-4}$ alkyl$)_2$.

Suitably, the third aspect of the present invention employs deterrents of formulae (VIII) or (IX)



(VIII)



(IX)

where $R^{21}$ represents hydrogen, $C_{1-4}$ alkyl or $C_{2-4}$ alkenyl, $R^{22}$ represents $C_{1-4}$ alkyl, $C_{1-4}$ alkoxy, hydroxy, $CO_2C_{1-4}$ alkyl, $CON(C_{1-4}$ alkyl$)_2$ or $C_{1-4}$ alkyleneCON($C_{1-4}$ alkyl$)_2$, $R^{23}$ represents $C_{1-4}$ alkyl or $C_{2-4}$ alkenyl, q is 1 or 2, and r is 1 or 2.

Preferred deterrents employed according to the third aspect of the present invention are one or more of eugenol (4-allyl-2-methoxyphenol), limonene (4-isopropyl-1-methyl-cyclohex-1-ene), *DEET* (N,N-diethyl-m-toluidine), *DEPA* (N,N-diethyl-phenyl-acetamide), *DMP* (dimethylphthalate) and thymol (5-methyl-2-isopropylphenol).

The above described deterrents are generally olfactive, volatile compounds. However, according to a fourth aspect of the present invention a non-volatile deterrent may be used in a composition according to the present invention. Suitably, a non-volatile deterrent for use in the present invention comprises a tetranortriterpenoid, such as azadirachtin or the like. A non-volatile deterrent of this type may be advantageous in facilitating preparation of a composition according to the present invention, for example facilitating incorporation into a molluscicidal pellet or the like.

Preferably, a deterrent employed in the present invention is one or more of a monohydric $C_{6-10}$ alcohol or a compound of formula (I), (III), (IV) or (VI). Preferred deterrents for use in the present invention include any of eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), octan-1-ol, borneol (endo-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol), camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one), sulcatol (6-methyl-5-hepten-2-ol), sulcatone (6-methyl-5-hepten-2-one), and D-3-carene (3,7,7-trimethylbicyclo[4.1.0]hept-3-ene).

The most preferred deterrents for use in the present invention include any of borneol (*endo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol), camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one), sulcatol (6-methyl-5-hepten-2-ol), sulcatone (6-methyl-5-hepten-2-one), and D-3-carene (3,7,7-trimethylbicyclo[4.1.0]hept-3-ene).

A deterrent employed in the present invention is suitably employed against invertebrates which are themselves important natural control agents for a number of crop pests, including molluscs. In particular, these non-target invertebrates can include insects, such as beetles, especially carabid beetles or the like.

Further ingredients which may be present include proteins, carbohydrates or the like, or surfactants, binders or the like. Such further ingredients may be bait materials (such as, for example, a yeast extract), which are attractive to slugs, or materials which act as foods for slugs.

Generally, a composition according to the present invention further comprises at least one inert carrier. The term "inert carrier" as used herein refers to a carrier suitable for use in molluscicidal compositions, which does not substantially adversely interact with the deterrent or molluscicide, or any other ingredients. The "inert carrier" may have a positive effect in that it may itself be attractive to slugs. An example of such a carrier is a starch, which is also a food for slugs.

Typically, inert carriers suitable for use in compositions according to the present invention comprise materials that can be intimately mixed with the deterrent and molluscicide suitable for use according to the present invention, together with any other optional ingredients which may be present. Intimate mixtures of the molluscicide, deterrent and one or more inert carrier, can suitably be shaped into conventional forms, such as pellets, blocks, pads or tapes, for use in accordance with the present invention. When pellets are used in accordance with the present invention, they are generally of a size of the order of substantially 2 by 5 mm.

There is further provided by the present invention a pellet comprising a molluscicidal composition substantially as herein before described, and optimally further comprising one or more inert carriers for the deterrent and molluscicide.

According to the present invention there is further provided use of a molluscicidal composition substantially as herein before described in destroying molluscs, and in particular destroying slugs, such as slugs of the species *Deroceras reticulatum*.

The present invention further provides use of a molluscicide in destroying molluscs, wherein the molluscicide is used in combination with at least one deterrent for non-target invertebrates. This use according to the present invention is suitably employed in destroying slugs substantially as described above.

The present invention further provides a method of destroying molluscs, which method comprises providing molluscs with a molluscicidal composition substantially as herein before described, and allowing the molluscs to ingest the molluscicidal composition.

There is also provided by the present invention a method of treating an environment with a molluscicidal composition substantially as herein before described, so as to effect substantial eradication of molluscs from the environment. Typically, the environment treated by a composition according to the present invention (referred to below as a treatment environment) is an arable area or pasture, a glasshouse, an area of ornamental plants or forestry seedlings or the like. Alternatively a composition according to the present invention may be employed in treating domestic or public gardens so as to substantially eradicate molluscs therefrom.

There is further provided by the present invention a method of deterring non-target invertebrates (and in particular the carabid beetle), from ingesting a molluscicide which has been employed in a treatment environment as described above, which method comprises treating the environment with a molluscicidal composition substantially as hereinbefore described, whereby the deterrent is present in sufficient quantity to substantially preclude ingestion of the molluscicide by the non-target invertebrates.

According to the present invention there is further provided a process of preparing a molluscicidal composition, which process comprises mixing at least one molluscicide for target molluscs and at least one deterrent for non-target invertebrates, so that deterrent incorporated in the composition has substantially no effect on target molluscs feeding on the composition and substantially precludes feeding on the composition by the non-target invertebrates.

Suitably a process according to the present invention optimally further comprises mixing the deterrent and molluscicide with one or more inert carriers therefor. Appropriately a process according to the present invention further comprises shaping the deterrent and molluscicide, and optimally the inert carrier, into a pellet, suitable for ingestion by molluscs.

The present invention further provides use of a molluscicide in the manufacture of a molluscicidal composition substantially as herein before described. The use comprises mixing the molluscicide with at least one deterrent for non-target invertebrates, and optionally one or more inert carriers therefor.

There is also provided use of at least one deterrent for non-target invertebrates in the manufacture of a molluscicidal composition substantially as herein before described. The use comprises mixing the deterrent with a molluscicide, and optimally further mixing with one or more inert carriers therefor.

According to yet a further aspect of the invention there is provided, in combination (for use in destroying molluscs):

at least one molluscicide for target molluscs; and

at least one deterrent for non-target invertebrates, whereby feeding target molluscs are substantially unaffected by the deterrent and non-target invertebrates are substantially precluded from feeding on the molluscicide by the deterrent.

The present invention will now be further illustrated by the following Example, which does not limit the scope of the invention in any way.

<u>Example</u>

<u>Electrophysiological screening</u>

<u>Electroantennogram</u>

The electroantennogram (EAG) equipment employed is illustrated in Figure 1, where (1) represents a microscope, (2) represents a stimulus pipette, (3) represents an applicator, (4) represents a stimulus controller, (5) represents an operating computer, (6) represents a start pedal, (7) represents an antennal preparation, (8) represents a manipulator and (9) represents an amplifier.

A carabid, partially anaesthetised through chilling, was placed under a microscope (1) and a single antenna removed by cutting at the base where it enters the head. An electrode was then inserted into the base of the antenna before being earthed, this serving as the “indifferent” or “reference” electrode.

The extreme tip of the antenna was removed before several sensillary hairs were positioned into the tip of the "recording" electrode using "leitz" micromanipulator (8). The recording glass electrode had previously been trimmed to be a tight fit over the tip of the antenna to prevent Ringer solution from leaking out over the preparation, causing background instability and interference. Both electrodes consisted of Ag/AgCl wire contained in glass microelectrodes and partially filled with Rhodnius Ringer solution.

A continuous stream of purified air was passed over the preparation at a rate of 1L/min. This was both generated and maintained by stimulus controller (4). Test chemicals were then introduced into this air stream for a 0.5 second period and subsequently delivered to the preparation. This was achieved by blowing air over a strip of paper, on which 10 μL of test chemical had previously been dosed. The filter paper was contained within stimulus pipette (2) which was then positioned into the centre of the air stream and test chemicals blown over antennal preparation (7). Before the chemicals were applied to preparation (7), hexane solvent was delivered to determine that no activity was induced by the solvent, therefore, signalling that any subsequent activity produced was a direct result of the test chemical.

An internal standard of 5% dimethyl trisulfide was also randomly applied throughout the course of screening, to eliminate the possibility of de-sensitisation of the preparation with time.

Taste Recordings

Non-volatile compounds were applied to gustatory chemoreceptors located on the maxillary and gustatory palps of the carabid. The test chemical was incorporated into an electrolyte in the recording electrode before being positioned over several sensillae. Any subsequent activity was stored onto computer through a specialised "delayed trigger" IADC interface box. Recordings from these sensillae produced coded responses comprising individual compound APs.

## Feeding Trials

### Maintenance of test animals

Carabid beetles were maintained in a controlled environment at 15°C, with a dark to light ratio of 16 hours dark to 8 hours light. Prior to feeding trials, the beetles were fed on a single blowfly maggot before being starved for a period of 5 days.

Slugs were also maintained in a controlled environment 15°C, 16 hour dark : 8 hour light ratio. Before testing the slugs were fed *ad lib* on potato and then starved for 24 hours.

### Filter paper feeding trials

Pre-starved carabid beetles were placed in uncovered glass arenas (20cm diameter) containing two pieces of 2.5cm diameter filter paper, previously coated in 50% maggot serum. One of these paper discs was then dipped in 0.1mg/ml test chemical while the other remained untreated serving as the control. Before being placed into the arena, the filter paper was left for a period of 20 minutes to allow the solvent (n-hexane) to evaporate, each test had 10 replicates. The discs were then placed at opposite ends of the dish and moistened with distilled water. After a period of 24 hours the papers were removed and the area consumed calculated using image analysis software.

### Slug feeding bioassays

Methiocarb-free slug pellets were placed in numbered positions on a glass plate, oven dried at 50°C and weighed. Six pre-weighed pellets were then placed in each of fifteen 9cm plastic petri dishes on two filter papers (Whatman No. 1). Each pellet was dosed with 1µL hexane solvent, thus serving as the control. The petri dishes were placed in the control environment room and the filter papers moistened with distilled water. One slug was placed in each dish and a piece of netting attached over the top to contain the slug but prevent build up of vapour from the volatile test material. After 24 hours the slugs were removed and the pellets replaced on the glass plate and re-dried. The dry weight of the pellet consumed by the slug during the test was then calculated by difference.

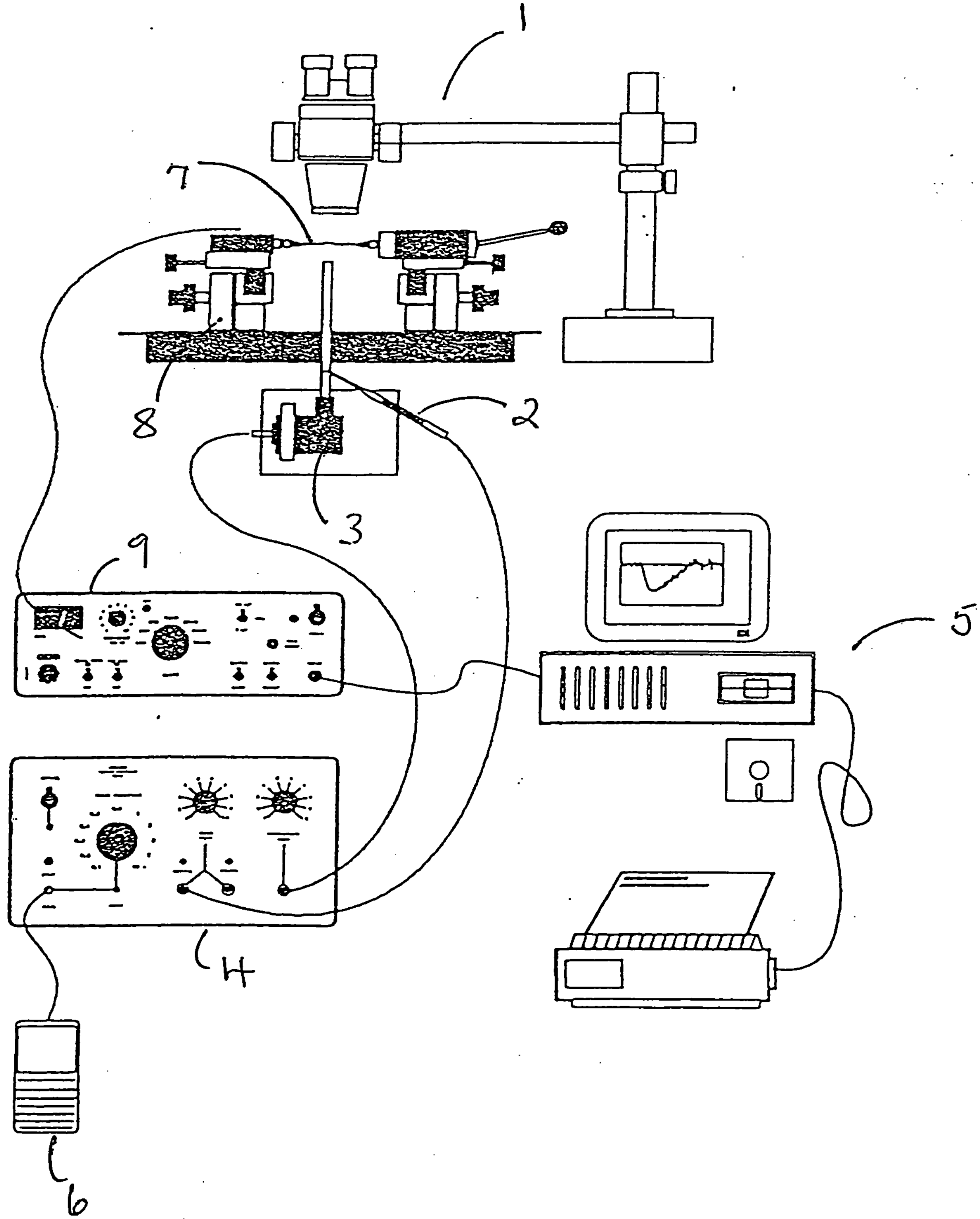
## Results

The following test compounds induced activity when screened on ten different preparations - camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one), borneol (*endo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol), octan-1-ol, eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), β-myrcene (7-methyl-3-methylene-octa-1,6- diene), sulcatol (6-methyl-5-hepten-2-ol), sulcatone (6-methyl-5-hepten-2-one), eugenol (4-allyl-2-methoxyphenol), limonene (4-isopropyl-1-methyl-1-cyclo-hexene), *DEET* (N,N-diethyl-m-toluidine), *DMP* (dimethylphthalate), α-pinene (2,6,6-trimethylbicyclo[3.1.1]hept-2-ene), D-3-carene (3,7,7-trimethylbicyclo[4.1.0]hept-3-ene) and azadirachtin.

In the filter paper feeding trials the following compounds were tested for deterrent activity, and as such were identified as particularly useful deterrents for the present invention - borneol (*endo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol), camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one), sulcatol (6-methyl-5-hepten-2-ol), sulcatone (6-methyl-5-hepten-2-one), and D-3-carene (3,7,7-trimethylbicyclo[4.1.0]hept-3-ene).

Borneol (*endo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol), octan-1-ol, sulcatol (6-methyl-5-hepten-2-ol) and sulcatone (6-methyl-5-hepten-2-one) were also further tested in the slug feeding bioassays. None of these compounds substantially affected the slug feeding behaviour, as illustrated in Figures 2 and 3. In particular, sulcatol was tested at concentrations of 0.01, 0.1 and 1.0mg/ml and no detrimental effects on the slug feeding behaviour were seen.

FIGURE 1



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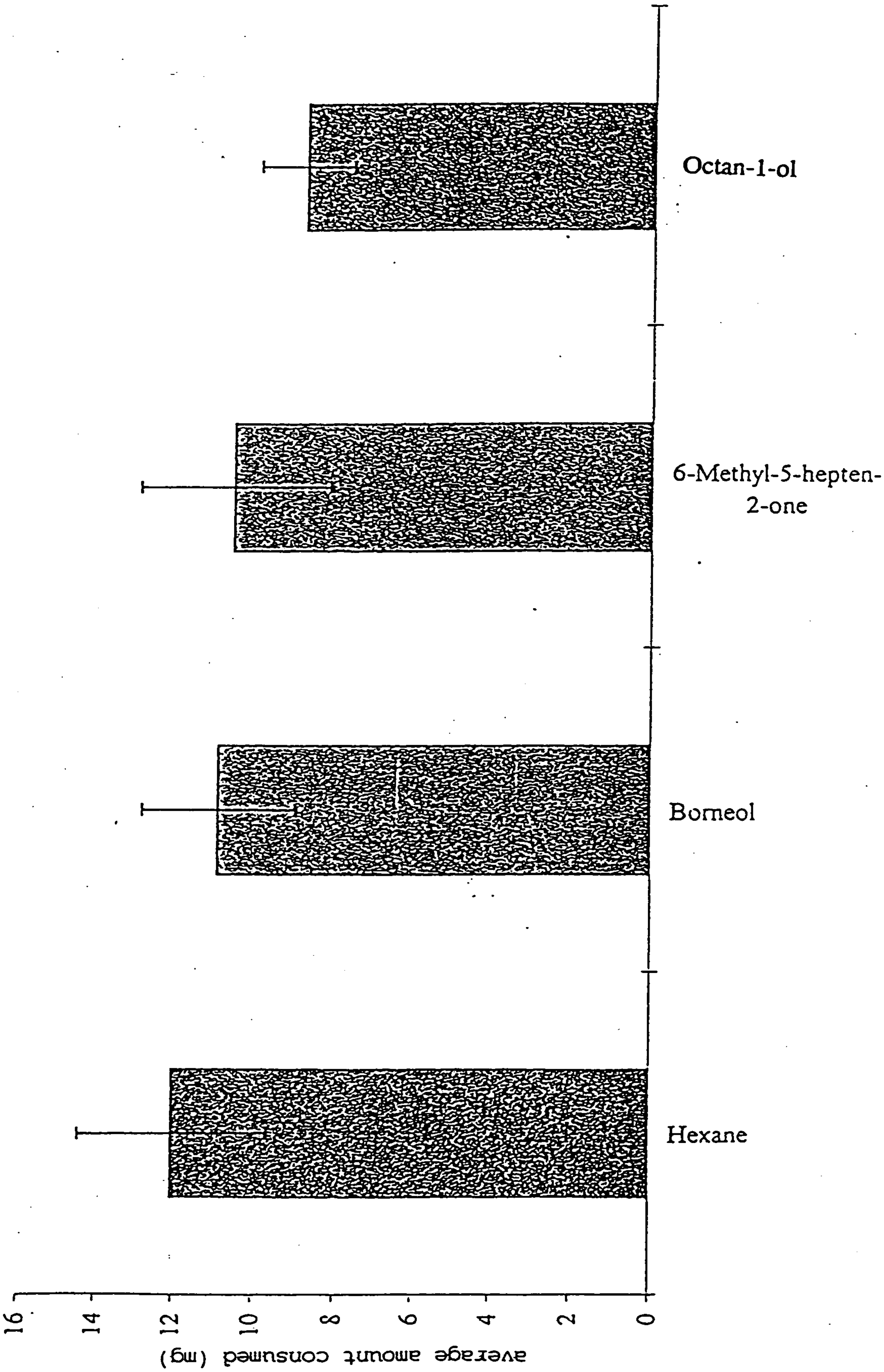
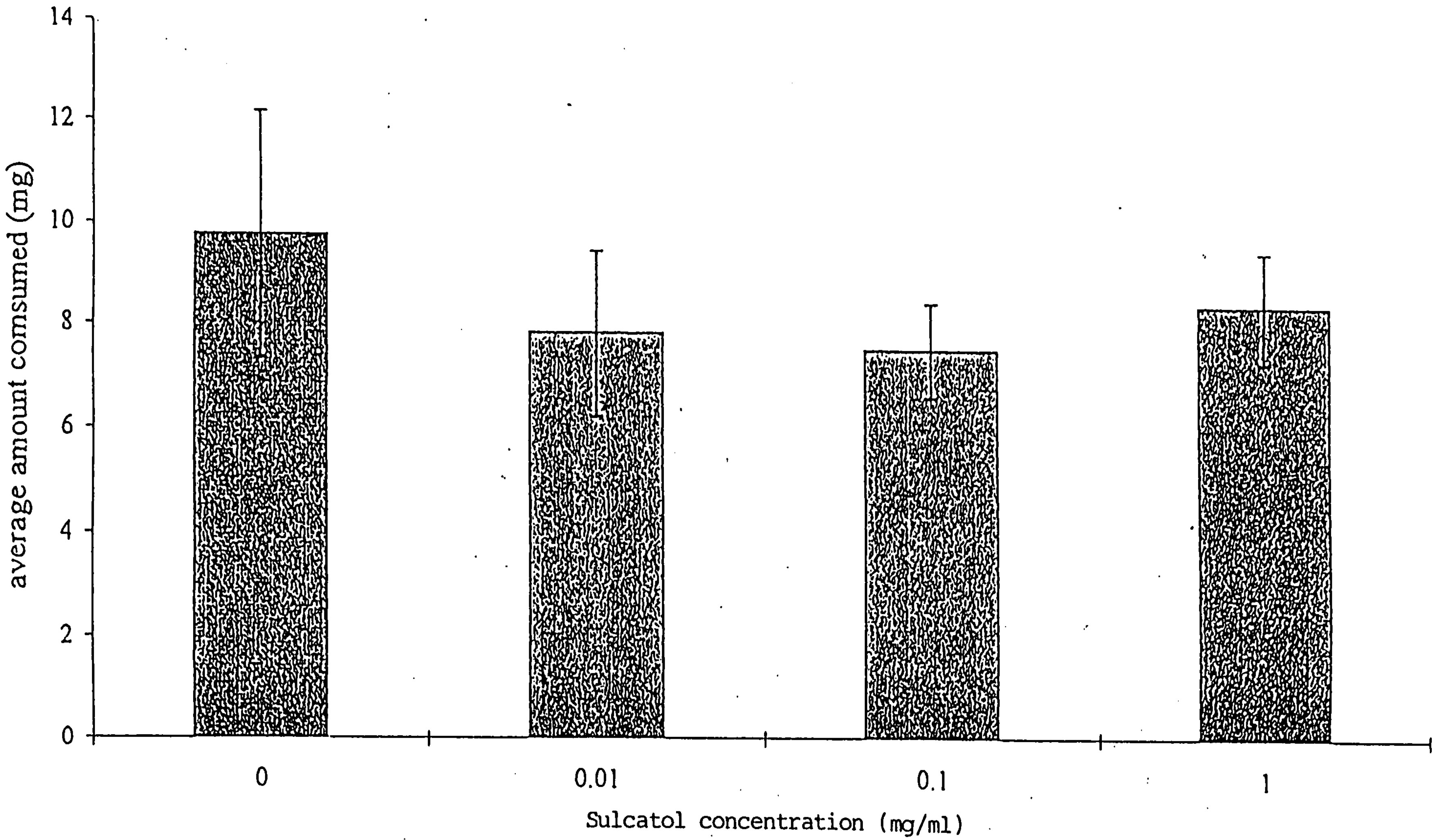

Octan-1-ol
6-Methyl-5-hepten-2-one
Borneol
Hexane
0
2
4
6
8
10
12
14
16
average amount consumed (mg)

FIGURE 2

FIGURE 3


average amount comsumed (mg)
14
12
10
8
6
4
2
0
0
0.01
0.1
1
Sulcatol concentration (mg/ml)